Final Report

Experimental analysis of the context-dependent effects of early life-stage PCB exposure on *Rana sylvatica*.

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Submitted by:
William J. Resetarits, Jr.
Associate Professor
Department of Biological Sciences
Old Dominion University
Norfolk, Virginia 23529

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Introduction

Several prior studies have investigated the effects of exposure to polychlorinated biphenyls (PCBs) on the survival, growth, and development of larval amphibians in a laboratory context (e.g., Reeder et al. 1998; Rosenshield et al. 1999; Gutleb et al. 2000; Savage et al. 2002). In addition, recent studies sponsored by the U.S. Environmental Protection Agency (EPA) have been undertaken to assay the potential effects of PCBs on amphibians in or captured in the floodplain of the Housatonic River in Berkshire County, MA (Weston 2000). The results of these latter studies have not been reported to date. All the above studies, however, leave major gaps in our ability to translate individual-level effects into population-level impacts on amphibians. To address one of the most significant of those gaps -- the effects and interactions of PCB exposure and density-dependence -- we undertook a field experimental study of the growth and development of the offspring of amphibians that had been exposed to varying concentrations of PCBs in the Housatonic River floodplain.

Laboratory studies have documented effects of PCB exposure on the survival, growth, and development of larval amphibians under certain exposure conditions (e.g., Reeder et al. 1998, Rosenshield et al., 1999; Gutleb et al. 2000; Savage et al. 2002). In addition, the recent EPA-sponsored studies are attempting to assess: (a) use and reproductive activity by adult frogs in a limited number of temporary ponds in the Housatonic River floodplain with varying concentrations of PCBs in sediments; and (b) amphibian reproductive success and development (as measured in the laboratory) from laboratory spawned eggs of sexually mature frogs captured from Housatonic River floodplain ponds with a range of sediment PCB concentrations (Weston 2000). However, these and most toxicity studies do not consider the extent to which PCB exposure, in the context of existing natural stressors and other influences in the environment, may ultimately affect the dynamics and viability of amphibian populations.

The question of how multiple stressors combine to impact populations of a species is not a question unique to the Housatonic River system or to studies of wildlife toxicology in general. It is a question that has been the focus of considerable basic ecological research, much of which has used amphibians as a model system (see summaries in Wilbur 1997; Werner 1998). The majority of this research has focused on interactions among biotic factors, primarily density-dependence, interspecific competition, and predation (e.g., Alford and Wilbur 1985, Morin 1983, Fauth and Resetarits 1991, Resetarits and Fauth 1998). Abiotic factors such as hydroperiod, pH, and the presence of chemical stressors (e.g., Wilbur 1987, Rowe and Dunson 1994, 1995, Diana et al. 2000, Relyea 2001, Boone and Semlitsch 2002) have also been integrated to provide a more complete understanding of how multiple stressors can influence individual and population-level responses. The singular characteristic of this body of work is the use of a rigorous experimental approach to understand the effects of multiple stressors and their interactions (Wilbur 1997, Werner 1998, Resetarits and Fauth 1998). Thus, the amphibian model system has itself become a model for the use of the most rigorous scientific approaches in ecological research.

How do density and PCB exposure level affect survival and growth of larval *Rana sylvatica*, and is there an interaction between these two factors in determining larval success? We addressed this question in an *in situ* enclosure experiment examining three levels of density and three levels of early life-stage PCB exposure. Larval amphibians may acquire PCBs through various pathways, including direct contact with and ingestion of sediments during their grazing on periphyton and phytoplankton, ingestion of food, dermal absorption of dissolved PCBs in water (although this is likely a minor pathway because PCBs exhibit very low solubility in water [Johnson et al. 1999]), and maternal transfer of PCBs into the lipid-rich component of the eggs. In the early larval period, the maternal transfer of PCBs is likely to be the primary source of

exposure and thus the most important pathway for early life-stage development. Accordingly, we used PCB concentrations in hatchlings as a reflection of that exposure pathway. Our primary concern in this experiment is whether the output and quality of metamorphs from pond enclosures are significantly affected by such maternally transferred PCB concentrations in the context of natural variations in conspecific density and other natural stressors acting on the population. This is the first study to examine the effects of such PCB exposure on larval performance in a natural environmental context.

Methods

The wood frog, *Rana sylvatica*, is the most widespread and abundant anuran species observed in the preliminary surveys of the Housatonic River floodplain (Kline 1999). It is also highly tractable in enclosure experiments (e.g. Wilbur 1972, 1976) and, because of its short larval period, provides efficient estimates of the effects of early larval PCB exposure. The short larval period should also magnify the importance of maternal transfer relative to other forms of larval exposure, which may be more important in species with extended larval periods. *Rana sylvatica* breed in early spring in temporary to permanent, usually fishless ponds. Eggs are laid in communal nest sites comprised of tens to hundreds of clutches. Hatching occurs in a few days to two weeks depending on temperature. Metamorphosis can occur in as little as six weeks, again depending on temperature and density. Adult wood frogs are largely terrestrial and are seldom observed outside of the breeding season. Overwintering occurs in terrestrial sites.

Exposure to PCBs in adult *R. sylvatica* may occur via consumption of invertebrate prey and possibly via direct soil contact during overwintering or use of refuges. Adult frogs thus exposed accumulate PCBs in the liver and other tissues (Gillan et al.1998; Huang et al. 1999; Fontenot et al. 2000), and females pass on a portion of accumulated PCBs via the lipids supplied

to their eggs, as observed in organisms as diverse as salmon (Miller and Amrhein 1995) and a variety of other fishes (Russell et al. 1999), snapping turtles (Russell et al. 1999), and domestic chickens (Bargar et al. 2001). As noted above, these maternally transferred PCBs are likely to be a primary source of PCB exposure in the early larval period.

Rana sylvatica eggs were collected from five ponds in the Housatonic River floodplain on 26-28 April 2001 (Figure 1a-d). These five ponds, designated as ponds 1.1, 1.2, 6.6, 10.7, and 10.9[1], were the only floodplain ponds where we were able to locate sufficient numbers of eggs during April 2001 to support the experimental design. At least 21 clutches were collected per pond, with the actual number depending on the size of the communal nest.

Eggs were returned to a workspace at the General Electric facility in Pittsfield, which had been cleaned prior to initiating the study. Eggs were maintained until they hatched and grew large enough to be introduced into experimental enclosures to be placed in floodplain ponds (as discussed below). Larvae must be mobile before they are hardy enough for handling, counting and transport; this took approximately ten days from collection, or just under two weeks from oviposition. Eggs were held one clutch per Rubbermaid container and clutches from different source ponds were interspersed by rows on the workspace floor so as to equalize initial conditions for clutches from all source ponds. On 1 May, samples of 200 hatchlings (larvae emerged from egg jelly but not fully mobile) from each of 21 clutches from each pond (4200 total/pond) were collected. These samples were combined into five composite samples (one from each of the five ponds), and these composite samples were transferred to an analytical laboratory for analysis of PCBs.

The results of these analyses are presented in Table 1 (second and third columns), along with the arithmetic mean concentrations of PCBs in the sediments from the same five source

[1] The number to the left of the decimal indicates the EPA tile map number and the number to the right of the decimal indicates the pond number within that tile.

ponds (sixth column) based on sampling conducted prior to this study. The analytical results from the hatchlings showed PCB concentrations (quantified as Aroclor 1260) ranging from 0.257 to 11.2 parts per million (ppm). Based on the results of these analyses and the number of available eggs from each pond, three ponds (ponds 10.7, 6.6, and 1.1) were chosen as sources for the experimental animals. These source ponds were selected to have three distinct PCB concentrations based on the hatchling samples; those concentrations were designated as very low (VL = 0.899 ppm – from source pond 10.7), low (LO = 3.28 ppm – from source pond 6.6), and high (HI = 11.2 ppm – from source pond 1.1). Pond 10.9 (with 0.257 ppm PCBs in the hatchlings) was not used as a source pond because not enough eggs were collected from that pond to accommodate the original experimental design, and pond 1.2 (with 8.5 ppm PCBs in the hatchlings) was not selected in order to have three distinct PCB concentration levels. Mortality of eggs, hatchlings, and larvae was negligible for all source ponds.

The experiment utilized a full-factorial, randomized complete block design, crossing three levels of PCB concentrations and three levels of initial larval density, each replicated once within each of two ponds (blocks), for a total of 18 experimental units (3 x 3 x 2 =18), as shown on Figure 2. We used this blocked design to account for possible variation between ponds. PCB concentrations were VL, LO, and HI, as defined above. These varying concentrations were used to test tested the null hypothesis that hatchling PCB concentration had no effect on measures of larval performance. Initial densities were 200, 400, and 800[2] larvae per enclosure (approximately 67, 133, and 267 per m^2). These density manipulations were used to test the null hypothesis that increasing initial density had no effect on the performance of larval populations. Under this hypothesis, lack of an effect or a negative effect would indicate the presence of density-dependence, while a significant positive effect would indicate that population-level

[2] A geometric progression centered on estimated modal density provides the best estimate of the form of the density-dependence curve when only three densities are used.

performance is positively associated with increasing initial density. The applied densities effectively cover the most likely range of larval wood frog densities, although both higher and lower densities occur in natural ponds.

Counting of the laboratory-hatched larvae began on 7 May and was completed on 8 May. Larvae were randomized in groups of five into sets of 200 larvae each, as required for the experiment (42 such sets), plus additional backup sets. Sets were then randomly assigned to the appropriate enclosures within each pond. Each set of 200 larvae was comprised of individuals from a minimum of 20 clutches of each PCB level. These methods assured that an equivalent random sample of the available genetic diversity was present within each enclosure and within each concentration of PCBs. This careful quality control also allowed extremely accurate assay of experimental effects by reducing error variance.

In addition, as larvae were being counted for introduction into the experimental enclosures, a second set of composite samples (referred to as larval samples) was generated for four of the five source ponds (excluding pond 10.9), frozen, and sent to the laboratory (on 14 May) for PCB analysis. The results of these analyses are also included in Table 1 (fourth and fifth columns). However, the earlier (hatchling) concentrations alone were used to assign ponds to treatments because these samples better reflect maternal transfer and early life-stage exposure than do the later (larval) concentrations, due to growth, metabolism, and exogenous feeding.

The 18 experimental enclosures were then established in two adjacent temporary ponds on the Housatonic River floodplain – pond 6.5 (designated "Upper") and pond 6.6 (designated "Lower") (Fig. 1c). These two ponds were used for the experiment because they supported natural populations of wood frogs and held water longer than most other ponds on the floodplain, assuring adequate time to complete the experiment under the drought conditions existing at the time. These ponds had very low concentrations of PCBs in sediment (with means of 0.5 ppm in

pond 6.5 and 0.35 ppm in pond 6.6 – see Table 1) and had very similar physical characteristics. Enclosures measured 1 x 1 x 3 meters and were constructed of 1/16" nylon mesh. Enclosures had fixed side and bottom panels, but were open at the top to allow natural solar radiation and colonization by aquatic insects. Enclosures were arrayed in a single row of nine enclosures in each pond (Fig. 2). Water depth was allowed to vary naturally. Treatment combinations (initial density and PCB concentration) were randomly assigned to enclosures within ponds, and the sets of larvae (see above) were randomly assigned to the appropriate enclosures. Containers holding larval sets were distributed into the enclosures, assignments were double-checked, and only then were larvae released. Enclosures were checked every other day for integrity, water level, and signs of metamorphosis. The experiment began on 10 May 2001 and was terminated on 2-3 July 2001, with one block terminated on each day. Metamorphs began emerging in large numbers on 2 July and the decision was made to terminate the experiment because of declining water levels. All metamorphs and surviving tadpoles were collected and returned to the laboratory, where they were counted and weighed to the nearest 0.001g. All tadpoles (n=1059) were weighed on 6 July. Metamorphs were weighed beginning 3 July and all metamorphs were weighed by 10 July (n=774). Metamorphs were weighed only after their tails had fully resorbed. Surviving metamorphs and tadpoles were then returned to their natal ponds.

Statistical Analysis

Independent variables considered in the statistical analysis were pond (in which the enclosures were placed), initial larval density, and hatchling PCB concentration. Enclosure means (mass) and enclosure totals (number) formed the units of analysis. Response variables were mean metamorph mass, mean tadpole mass, number of metamorphs, and number of surviving tadpoles (Wilbur 1972, 1976, 1987; Morin 1983; Smith 1987). Mass data were log

transformed prior to analysis. Data from the experiment were analyzed using multivariate analysis of variance (MANOVA) on the vector of metamorph responses (mean number and mean mass of metamorphs) and tadpole responses (mean number and mean mass of tadpoles). MANOVA provides a single test criterion and test statistic for the effects of each independent variable on a set of correlated response variables. Wilk's λ was used as the test criterion for MANOVAs.

Since our design was limited to 18 experimental units, we had insufficient degrees of freedom to simultaneously test all main effects and the interactions between density and PCB concentration. We first tested for an interaction between density and PCB concentration. If there is an interaction between main effects in a factorial experimental, the interaction becomes the appropriate entity of interest; in the absence of interaction, a factorial experiment behaves as if two separate experiments had been done, each manipulating a single factor. This is the power of the factorial design. Our initial analysis detected no interaction between density and PCB concentration, so we proceeded with our analysis of the main effects. Since our multivariate response vector contains all of the response variables measured in our experiment, it provides a single, overall answer to the questions of the impact of each of the specific independent variables (pond, density, and PCB concentration) on our focal species. This analysis, however, is conservative by design, and so it is advisable to examine the component univariate ANOVAs as well. Thus, univariate ANOVAs were conducted on each of the component responses to determine the relative contributions to the overall variance. All tests used type III sums of squares and $\alpha = 0.05$. Data were analyzed using SAS for Windows release 8.0 (SAS Institute, Cary, North Carolina, 2000).

Results

Concentration of PCBs in composite hatchling and larval samples from the five breeding ponds in the Housatonic River floodplain showed no evidence of a relationship to PCB concentrations in the sediments of the breeding ponds themselves (hatchling - $F_{1,3} = 1.818$, $p = .27$; larval - $F_{1,2} = 1.13$, $p = .40$) (see also Table 1, Fig. 3). This lack of correlation suggests that females occurring at a given breeding pond are drawn from a variety of habitats across the floodplain (or even off the floodplain), and indicates that *R. sylvatica* may occur as widespread metapopulations or large subdivided populations on the floodplain, as seen in many other amphibians (see Alford and Richards 1999 and Marsh and Trenham 2001 for reviews). PCB concentrations in adult females (and consequently, in eggs) constitute an integration of exposures across the variety of habitats the female traversed over her lifetime. As expected, PCB concentrations in the offspring (assumed to be initially due primarily to maternal transfer) decline in concentration as larvae grow and develop. In our limited sample, sediment PCB concentration in the pond where egg laying occurred was a poor predictor of PCB concentrations in both hatchling and larval *R. sylvatica*.

The data for each of the 18 enclosures, including the number and mean mass of tadpoles and metamorphs for each, are summarized in Table 2. The key resulting comparisons are illustrated in Figures 4 through 6 and the results of the statistical analyses are provided in Table 3.

MANOVA revealed that the multivariate response vector of number and size of metamorphs and number and size of surviving tadpoles differed significantly between the two ponds in which the experiment was conducted (Table 3a, Figure 4). For both life stages (tadpoles and metamorphs), the difference derived from the fact that pond 6.6 showed a larger size of both metamorphs (0.284 ± .028 g; mean ± 1 SE) (Fig. 4) and surviving tadpoles (0.299 ±

0.023 g) (Fig. 4), compared to pond 6.5 (0.171 ± .011 g and 0.240 ± 0.009 g, respectively) (Table 3d, e; Fig. 4), while numbers of each life stage were not significantly different between ponds (Table 3b, c ; Fig. 4). This finding corresponds to the actual habitat selection between the two ponds by ovipositing *R. sylvatica*, which bred primarily in pond 6.6 in 2001 (*personal observation*).

In contrast, there was no evidence of a significant effect of either hatchling PCB concentration or density on the multivariate response vector described above (Table 3a, Figs. 5 and 6). Thus, there was no detectable overall effect of either density or PCB concentration on the performance of our experimental populations. Univariate ANOVAs also revealed no significant effects of either density or PCB concentration on metamorph responses (Table 3b,d, Figs. 5, 6). Density had a significant effect on both tadpole number and size, but these effects were in opposite directions, as would be predicted. That is, the number of surviving tadpoles paralleled the initial differences in densities (a positive relationship, Table 3c, Fig. 5), while the size of surviving tadpoles showed a negative relationship with initial density (Table 3e, Fig. 6). There was no overall net effect of initial density on tadpole responses (Table 3a). PCB concentration had a significant effect only on number of surviving tadpoles, as a result of the high relative survival in LO PCB enclosures compared to the VL and HI enclosures (Table 3c, Fig. 5).

Thus, overall survival and performance of metamorphs and tadpoles were not dependent on either density or PCB concentration. Within the range of densities and hatchling PCB exposure levels represented in our experiment, there was no evidence of a consistent positive or negative effect of either density or PCB concentration on factors that affect population regulation. It is important to emphasize that density effects are being viewed from a population perspective, so what the data indicate is that increasing larval density does not result in a positive

effect on population-level responses. Thus, density-dependence is acting to decrease the per capita survival and growth rates with increasing initial density, which is precisely what we would expect. The density responses in this experiment (Figs. 5, 6) reflect the typical patterns of density-dependent responses seen in larval anurans. In contrast, there was no evidence of a consistent effect of PCB concentration on larval performance. The only evidence of a significant effect of PCBs, in statistical terms, was an increase in tadpole survival in LO PCB enclosures relative to both VL and HI enclosures. This might be viewed as an artifact, but for the fact that four of the six highest values for tadpole survival were in LO enclosures, and these enclosures came from both ponds in which the enclosure were placed (two from pond 6.5 and two from pond 6.6) (see also Discussion).

Finally, the overall health of the experimental populations was qualitatively evaluated. All of the egg clutches maintained in the laboratory appeared healthy, regardless of source pond, more so than any collection of field-collected egg clutches we have seen for any species. We noted no appreciable mortality or behavioral or morphological abnormalities up through our counting process. Similarly, we noted no evidence of unusual pathology during the processing of the metamorphs and surviving tadpoles.

Discussion

The experiment detected no negative effects of PCB concentrations in the hatchlings (likely derived primarily from maternal exposure) on measures of performance (survival and growth) in larval *Rana sylvatica*. There were also no significant interactions between density and PCB concentrations, which would be expected if the effects of multiple stressors were additive or synergistic (Wilbur 1987; Fauth and Resetarits 1991; Relyea and Mills 2001). As previously noted, initial density had no significant net effect on overall performance of the larval

R. sylvatica. Given the structure of our null hypothesis, the lack of a significant positive relationship between initial density and output indicates that density-dependence is operating. Moreover, as illustrated in the figures, the responses to initial density showed the typical signal of density-dependence. While the number of surviving tadpoles showed a positive relationship with initial density, the number of metamorphs did not, and the size of surviving tadpoles (as well as metamorphs) showed the expected negative relationship with density (Figs. 5, 6). Thus, the larval anurans responded to initial density with decreasing per capita performance (although the effects were diluted by the fact that declining water levels prevented us from allowing all individuals to develop to metamorphosis -- which would tend to reduce the overall background variation). This serves to validate the sensitivity of the experiment, because density-dependence is one of the classic responses of larval anurans (e.g., Brocklemann 1969; Morin 1983; Alford and Wilbur 1985; Wilbur 1987; Fauth and Resetarits 1991); failure to detect density-dependence would suggest a lack of sensitivity in the experiment. In addition, the consistent and significant differences between the Upper and Lower Ponds (Fig. 4), reflected in both the MANOVA and individual ANOVAs, are further evidence of the power of the experiment to detect meaningful variation in larval and metamorph performance.

The only statistically significant positive or negative effect of PCBs on *R. sylvatica* was the increased number of surviving tadpoles in LO PCB enclosures relative to both VL and HI enclosures. As mentioned above, this finding does not appear to be an artifact. At the same time, this result cannot be causally attributed to PCBs because the lack of an exposure-response relationship implicates a factor other than PCB concentration in the enhanced performance of the LO PCB tadpoles (relative to the VL and HI groups). The most parsimonious explanation is the fact that that the LO PCB larvae came from source pond 6.6 and thus represent the “native” frog

population for the two enclosure ponds, suggesting some level of local adaptation. This issue cannot be resolved without further investigation.

Laboratory studies have demonstrated negative effects of PCBs on the survival, growth, development, time to metamorphosis, sex ratio, swimming performance, and other measures of health and performance of larval anurans under certain exposure conditions (e.g., Reeder et al. 1998; Rosenshield et al. 1999; Gutleb et al. 2000; Savage et al 2002). These effects, however, have occurred at the higher end of exposure levels, well above the measured PCB concentrations in the Housatonic River floodplain ponds that we reviewed and significantly higher than the concentrations measured in our hatchling and larval *R. sylvatica*. For example, Savage et al. (2002) found significant effects of exposure to PCB-contaminated sediments in *R. sylvatica* larvae in the laboratory (using only a single clutch, however), but these larvae were exposed to either 20 g or 40 g of sediments containing over 300 ppm PCBs and were found to have PCB body burdens of 22 ppm (for larvae that contacted the 20 g of contaminated sediments) or 128 ppm (for the larvae in contact with the 40 g of contaminated sediments). Although it is somewhat difficult to evaluate the meaning of varying the amount of sediment rather than the sediment concentration, it is clear that the PCB body burdens of larvae negatively affected in the Savage et al. (2002) experiment were much higher than the exposure levels we observed in hatchling *R. sylvatica* in our samples from the Housatonic River floodplain.

It should also be kept in mind that laboratory studies of amphibians that focus on a single stressor may either underestimate or overestimate the effects of contaminants on individuals when they are combined with other stressors, both natural and anthropogenic, in the natural environment (e.g., Cooke 1971; Kiesecker and Blaustein 1995; Long et al. 1995; Ankley et al. 1998; Verrell 2000; Kiesecker et al 2001; Davidson et al. 2001; Relyea and Mills 2001; Rowe et al. 2001; Boone and Semlitsch 2002). Such “context-free” studies shed little light on the impact

of stressors on populations or the communities they comprise (Marschall and Crowder 1996; Relyea and Mills 2001; Boone and Semlitsch 2002). Thus, it is critical to examine the effects of exposure in the context of natural environmental variation and species interactions. This study applies, in an experimental manipulation, one known (density) and one potential (PCBs) stressor against such a backdrop of natural variation. Included in the natural variation are fluctuations in temperature, water level, resources, and insect predation as a result of natural colonization of enclosures. This complex environment tests the organisms to a much greater degree than the environment of the laboratory, where only a single stressor is typically present.

Within this context, our data provide no evidence that early larval mortality and performance in natural *R. sylvatica* populations are negatively affected by hatchling PCB exposure within the range of levels represented in our study. This suggests that the early life stages of anurans like *R. sylvatica* may be relatively unaffected by these levels of PCB exposure. Recent studies have also suggested that amphibian populations may be relatively insensitive to considerable variation in early life stage mortality and performance, due to density compensation and the high fecundity and extended life span of adults (Vonesh and de la Cruz *in press*). This relative insensitivity has also been shown quite clearly for sea turtles (Crouse et al. 1987), fish (Rose et al. 2001), and a variety of other organisms whose adult stages are relatively long compared to the larval/juvenile stages (see Caswell 2000). Thus, in such circumstances, impacts on early life stages that would have the potential to significantly affect population level dynamics would be expected to be substantial and readily apparent in terms of effects on larval performance and survival. This is especially true when examined in the context of natural ecological interactions and abiotic stressors. This observation suggests that even the strongest effects observed in our study, the differences between the upper and lower ponds where the enclosures were placed, may be insufficient to impact local populations of *R. sylvatica.*

In our study, no effects of early life stage PCB exposure were in evidence, suggesting that such exposure via maternal transfer of PCBs at the levels represented in our study does not play a detectable role in the population dynamics of *R. sylvatica* in the Housatonic River floodplain. This finding is important because it indicates that the egg, hatchling, larval and metamorph stages are unlikely to be limiting in terms of population dynamics in this system, and that exposed females can positively contribute to population maintenance and growth. It also suggests that early life stage PCB exposure via maternal transfer at the levels observed in our five breeding ponds does not contribute to multiple-stressor, multi-stage impacts that could disrupt populations through their cumulative effects across a species life history (Marshall and Crowder 1996).

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Table 1. PCB concentrations in composite hatchling samples (approximately 1-2 weeks post-oviposition) and larval samples (approximately 3-4 weeks post-hatching) from five ponds and the mean (arithmetic) PCB concentration in the pond sediments from six ponds, measured at 0.15 m depth. All values are Aroclor 1260. There is no significant relationship between sediment PCB concentrations and hatchling or larval PCB concentrations (see text and Figure 3). Hatchling PCB concentrations were used to categorize the larvae for the experiment. Ponds 10.7, 6.6, and 1.1 were the source ponds for the larvae used in the experiment; experimental enclosures were set up in ponds 6.5 and 6.6.

Pond #	Hatchling PCB concentration (ppm)	Lipid-normalized hatchling PCB concentration (ppm)	Larval PCB concentration at 3-4 weeks post-hatching (ppm)	Lipid-normalized larval PCB concentration (ppm)	Sediment PCB concentration (ppm)
10.9	0.257	7.1	n/a	n/a	4.45
10.7	0.899	28.3	1.51	145.19	7.50
6.5	n/a	n/a	n/a	n/a	0.5
6.6	3.28	84.1	1.42	133.96	0.35
1.2	8.54	210.3	6.14	795.34	1.05
1.1	11.2	328.4	7.17	827.94	30.80

Table 2. Data summary. Pond abbreviations are "U"= upper, "L" = lower.

Enclosure	Pond	PCB	Initial larval Density (# per enclo-sure)	Mean meta-morph mass (g)	Meta-morph #	Mean tadpole mass (g)	tadpole #
6.5.9	U	HI	200	0.242	49	0.285	8
6.6.8	L	HI	200	0.386	28	0.421	6
6.5.5	U	HI	400	0.176	56	0.259	18
6.6.5	L	HI	400	0.418	21	0.351	12
6.5.4	U	HI	800	0.118	48	0.207	110
6.6.3	L	HI	800	0.248	53	0.285	35
6.5.1	U	LO	200	0.170	59	0.262	40
6.6.2	L	LO	200	0.314	55	0.308	27
6.5.6	U	LO	400	0.165	43	0.249	119
6.6.7	L	LO	400	0.171	29	0.253	104
6.5.3	U	LO	800	0.198	30	0.244	77
6.6.6	L	LO	800	0.167	24	0.197	219
6.5.7	U	VL	200	0.162	45	0.221	52
6.6.9	L	VL	200	0.284	38	0.254	26
6.5.8	U	VL	400	0.148	58	0.233	63
6.6.1	L	VL	400	0.300	35	0.357	18
6.5.2	U	VL	800	0.160	28	0.203	87
6.6.4	L	VL	800	0.273	50	0.263	37

Table 3. a). Multivariate analysis of variance for the response vector of mean metamorph number, mean surviving tadpole number, mean metamorph mass, and mean tadpole mass. Univariate ANOVAs are shown below for the four components of this response vector: b) metamorph number, c) tadpole number, d) metamorph mass, e) tadpole mass. Statistically significant results indicated by asterisks.

a)

Source	Wilk's λ	F	Num DF	Den DF	Pr > F
pond	**0.261**	**6.37**	**4**	**9**	**0.01***
density	**0.451**	**1.10**	**8**	**18**	**0.41**
PCB	**0.371**	**1.44**	**8**	**18**	**0.25**

b) metamorph number

Source	df	SS	MS	F	Pr > F
pond	1	382.7	382.7	2.12	0.1706
density	2	154.8	77.4	0.43	0.6603
PCB	2	23.4	11.7	0.07	0.9373
error	12	2161.3	180.1		

c) tadpole number

Source	df	SS	MS	F	Pr > F
pond	1	450.0	450.0	0.26	0.62
density	2	13823.4	911.7	3.97	0.05*
PCB	2	14347.4	7173.7	4.12	0.04*
error	12	20892.2	1741.0		

d) metamorph mass

Source	df	SS	MS	F	Pr > F
pond	1	0.197	0.197	18.37	0.0011*
density	2	0.048	0.024	2.24	0.1492
PCB	2	0.033	0.016	1.52	0.2572
error	12	0.128	0.011		

e) tadpole mass

Source	df	SS	MS	F	Pr > F
pond	1	0.033	0.033	7.31	0.02*
density	2	0.038	0.019	4.25	0.04*
PCB	2	0.022	0.011	2.40	0.13
error	12	0.054	0.005		

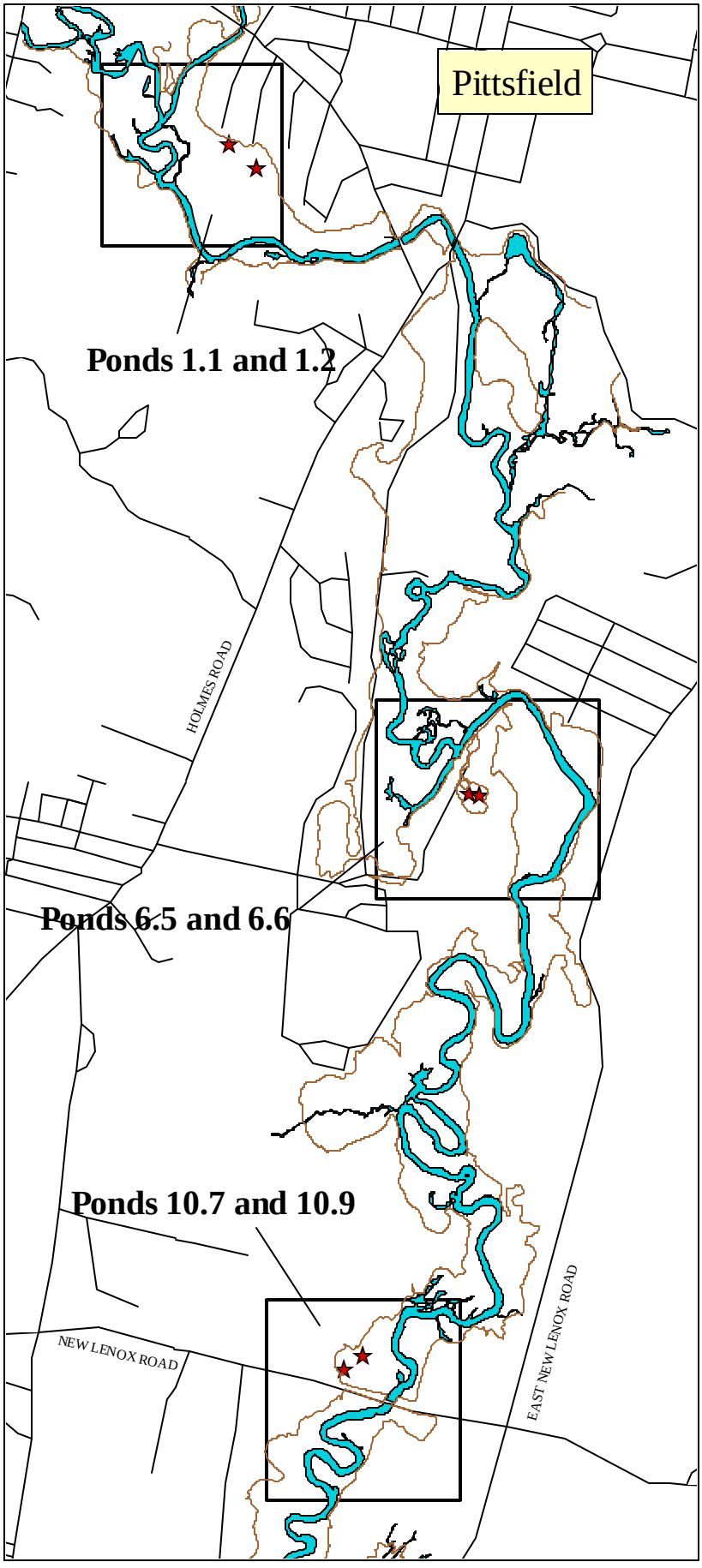

Figure 1a.

Frog Pond Locations

Housatonic River, MA

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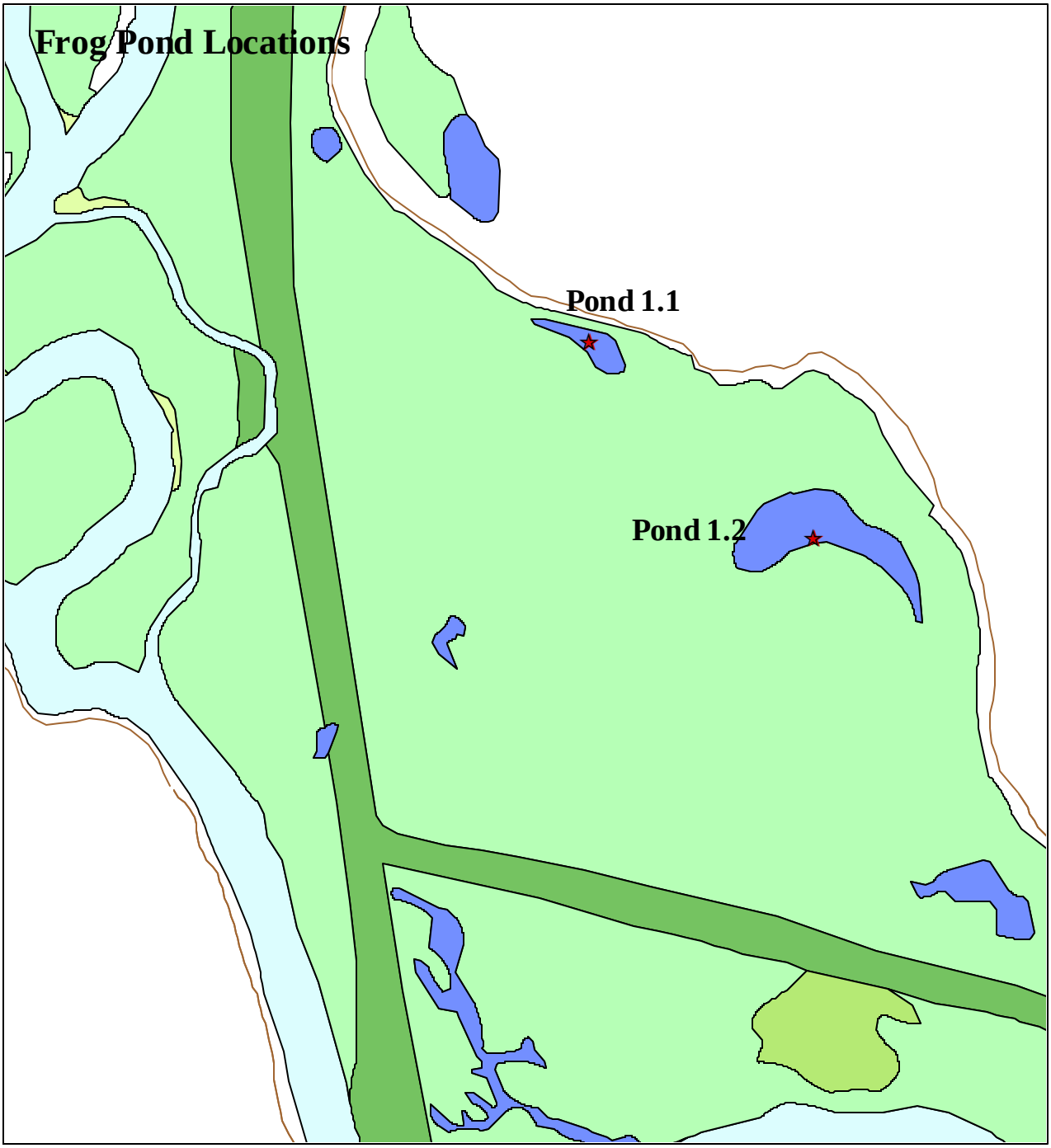

Figure 1b.

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Legend

Frog Egg Masses Collected
Enclosures

1 ppm isopleth (approximate extent of 10-year floodplain)

Landcover

LOW: Lacustrine, Open Water
PAB/UB: Palustrine, Aquatic Bottom/Unconsolidated Bottom
PEM: Palustrine, Emergent
PFO: Palustrine, Forested
PFO/EM: Palustrine, Forested/Emergent
PFO/SS: Palustrine, Forested/Scrub-Shrub
PSS: Palustrine, Scrub-Shrub
PSS/EM: Palustrine, Scrub-Shrub/Emergent
PUB: Palustrine, Unconsolidated Bottom
RAB: Riverine, Aquatic Bottom
ROW: Riverine, Open Water
SAND: Sand
UPLAND: Upland
WET_MEAD: Wet Meadow

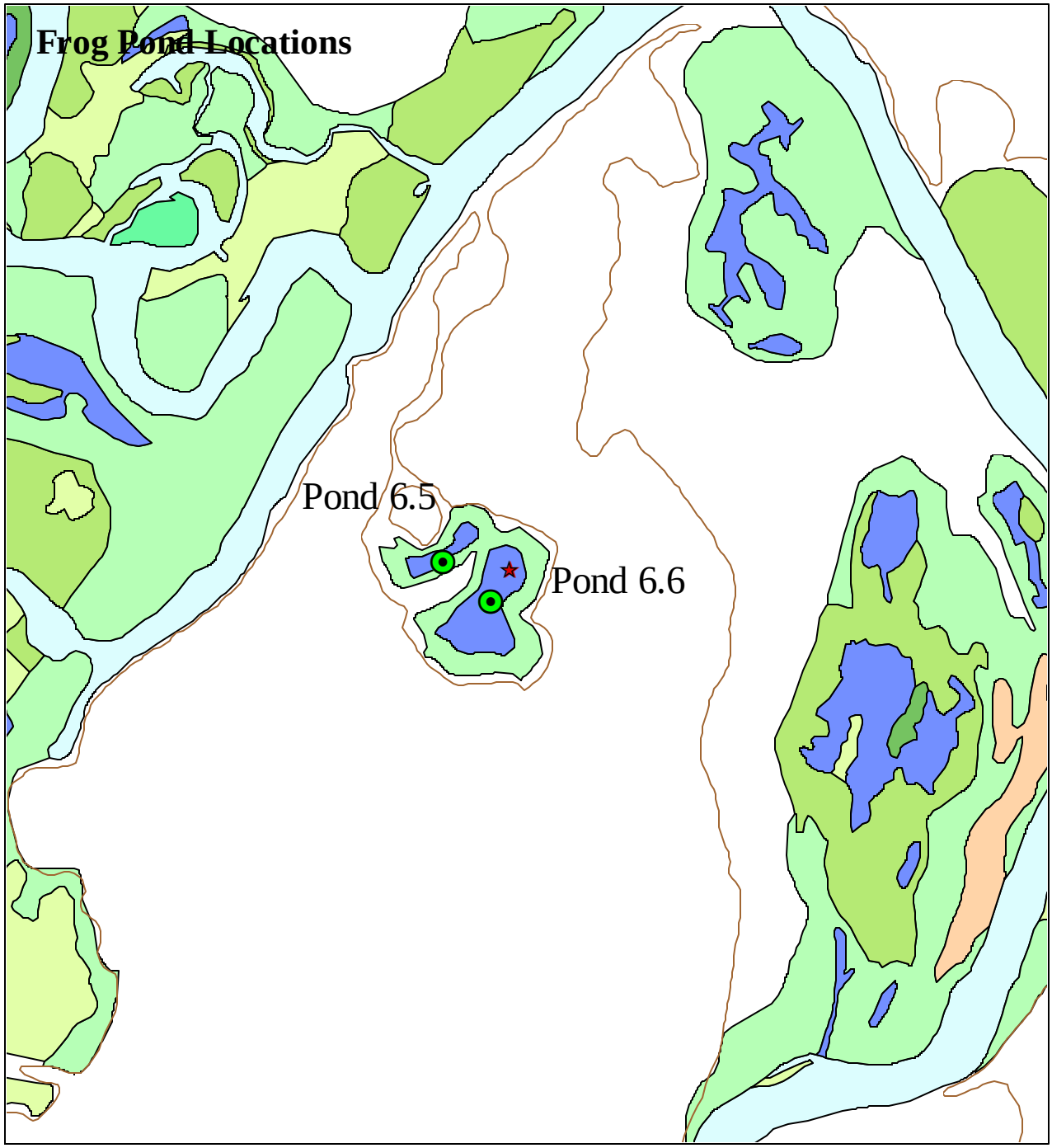

Figure 1c.

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Legend

- Frog Egg Masses Collected
- Enclosures
- 1 ppm isopleth (approximate extent of 10-year floodplain)

Landcover

- LOW: Lacustrine, Open Water
- PAB/UB: Palustrine, Aquatic Bottom/Unconsolidated Bottom
- PEM: Palustrine, Emergent
- PFO: Palustrine, Forested
- PFO/EM: Palustrine, Forested/Emergent
- PFO/SS: Palustrine, Forested/Scrub-Shrub
- PSS: Palustrine, Scrub-Shrub
- PSS/EM: Palustrine, Scrub-Shrub/Emergent
- PUB: Palustrine, Unconsolidated Bottom
- RAB: Riverine, Aquatic Bottom
- ROW: Riverine, Open Water
- SAND: Sand
- UPLAND: Upland
- WET_MEAD: Wet Meadow

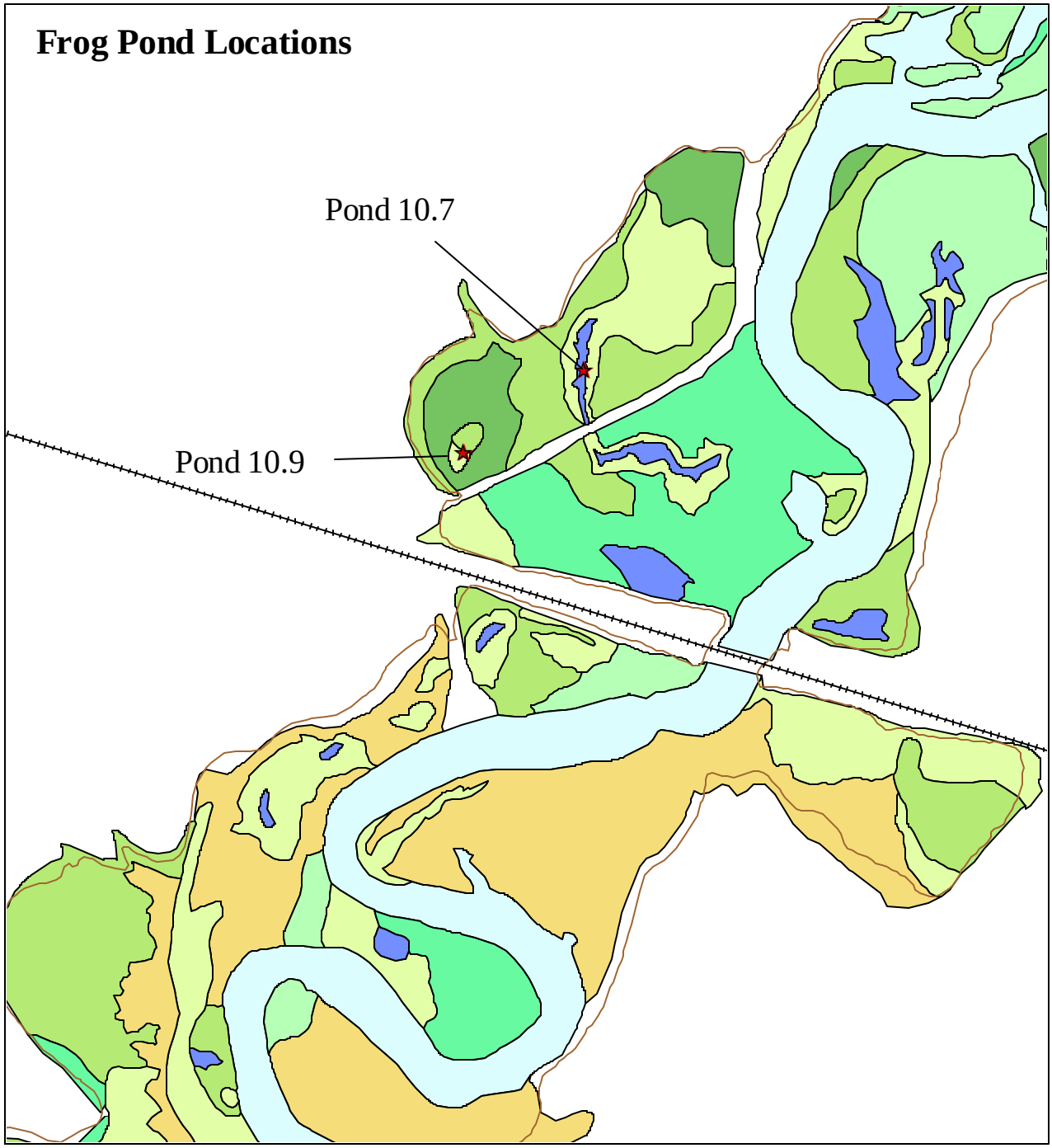

Figure 1d.

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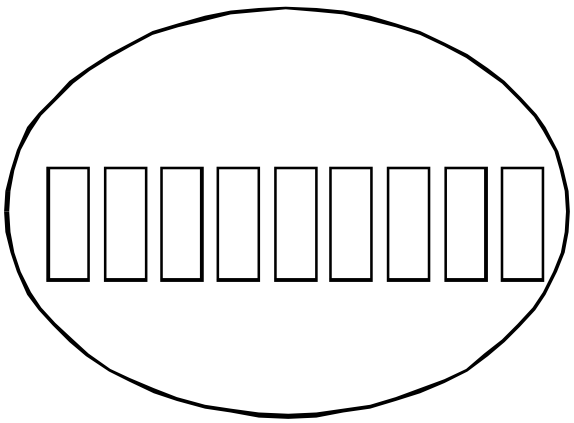
Legend

- Frog Egg Masses Collected
- Enclosures
- 1 ppm isopleth (approximate extent of 10-year floodplain)

Landcover

- LOW: Lacustrine, Open Water
- PAB/UB: Palustrine, Aquatic Bottom/Unconsolidated Bottom
- PEM: Palustrine, Emergent
- PFO: Palustrine, Forested
- PFO/EM: Palustrine, Forested/Emergent
- PFO/SS: Palustrine, Forested/Scrub-Shrub
- PSS: Palustrine, Scrub-Shrub
- PSS/EM: Palustrine, Scrub-Shrub/Emergent
- PUB: Palustrine, Unconsolidated Bottom
- RAB: Riverine, Aquatic Bottom
- ROW: Riverine, Open Water
- SAND: Sand
- UPLAND: Upland
- WET_MEAD: Wet Meadow



x 2 ponds

PCB concentration in hatchlings

Density	VL	LO	HI
800	N=2	N=2	N=2
400	N=2	N=2	N=2
200	N=2	N=2	N=2

Fig. 2. Schematic illustrating the experimental design. Matrix in the lower right is repeated once in each of two ND ponds.

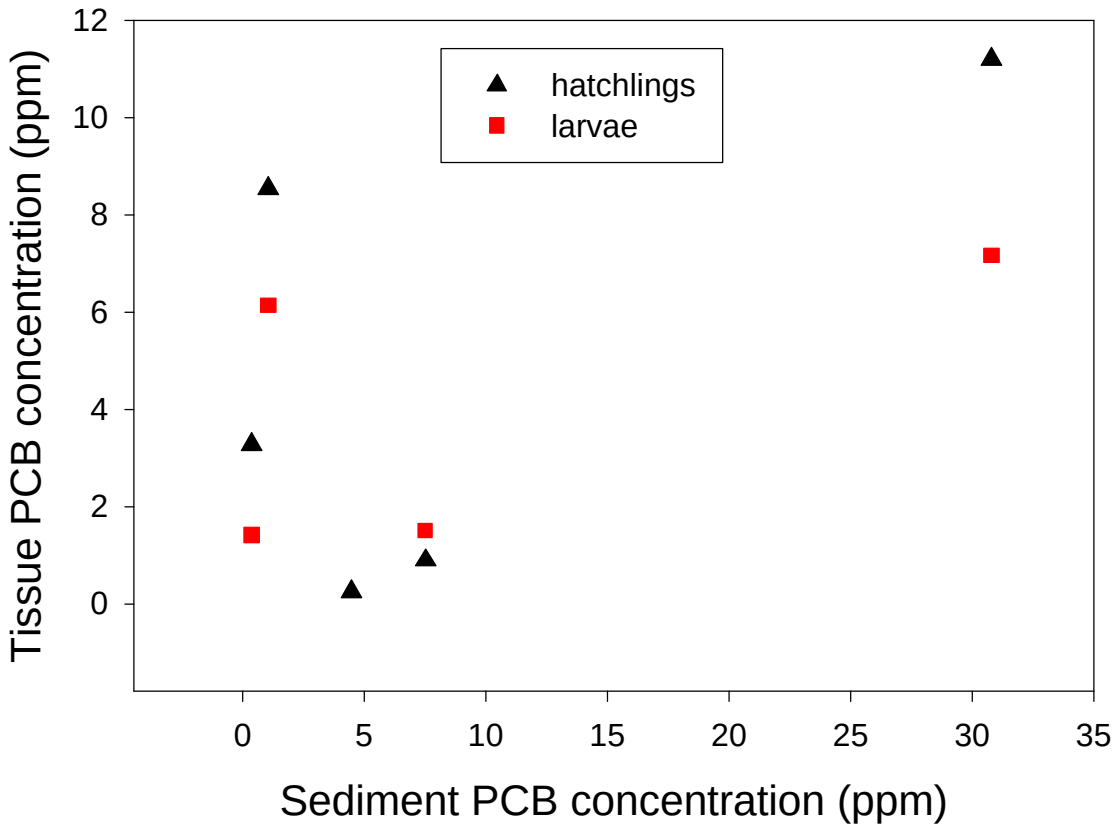


Fig.3. Relationship between hatchling and larval PCB concentrations and PCB concentrations in the sediments of the breeding ponds. Neither hatchling nor larval PCB concentrations show a significant relationship with sediment concentrations (see text).

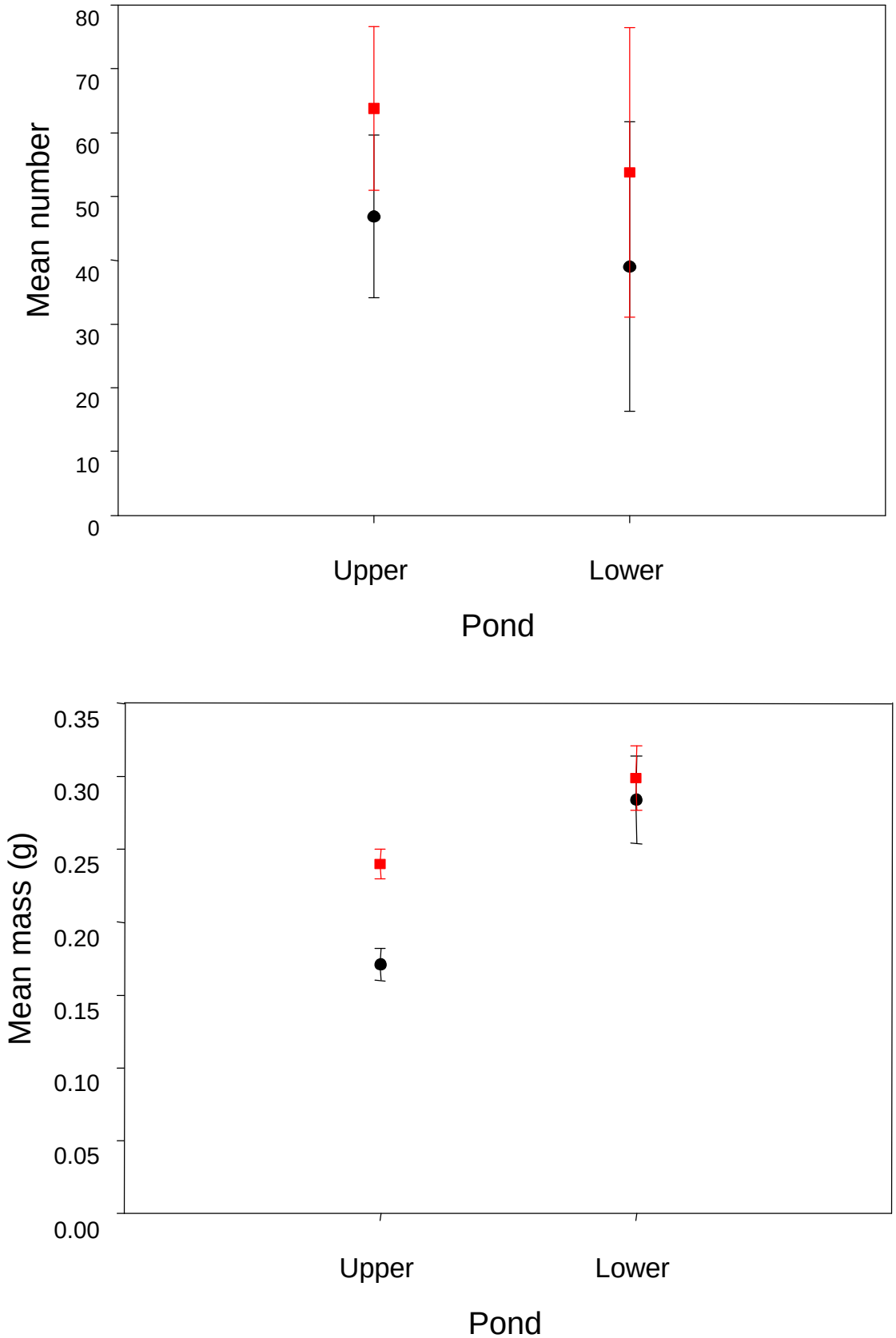


Fig. 4. Mean number (± 1 SE) (top) and mean mass (± 1 SE) (bottom) of metamorphs (black circles) and surviving tadpoles (red squares) in the two different ponds.

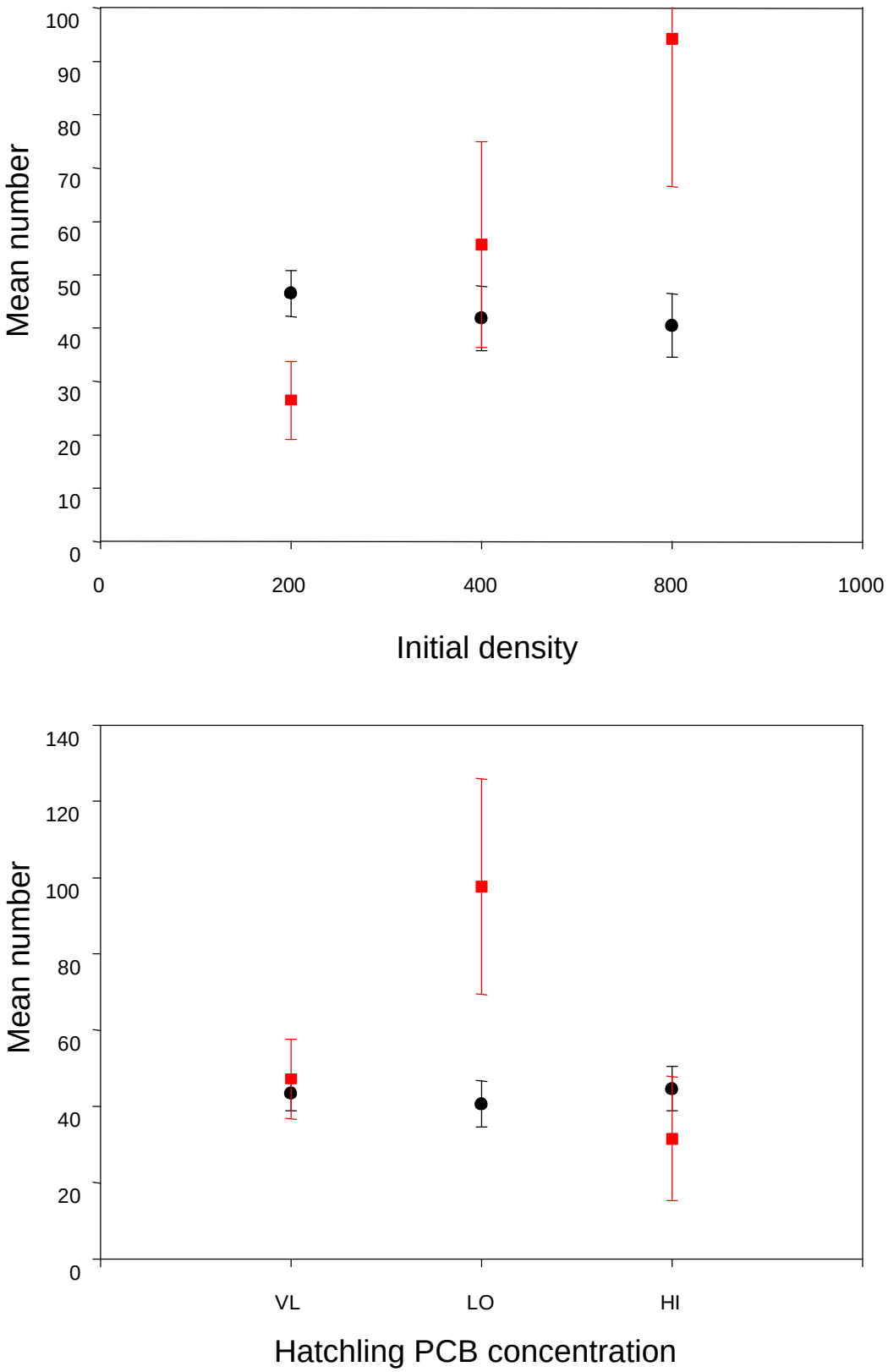


Fig. 5. Mean number (± 1 SE) of metamorphs (black circles) and surviving tadpoles (red squares) in response to initial density (top) and PCB concentration (bottom).

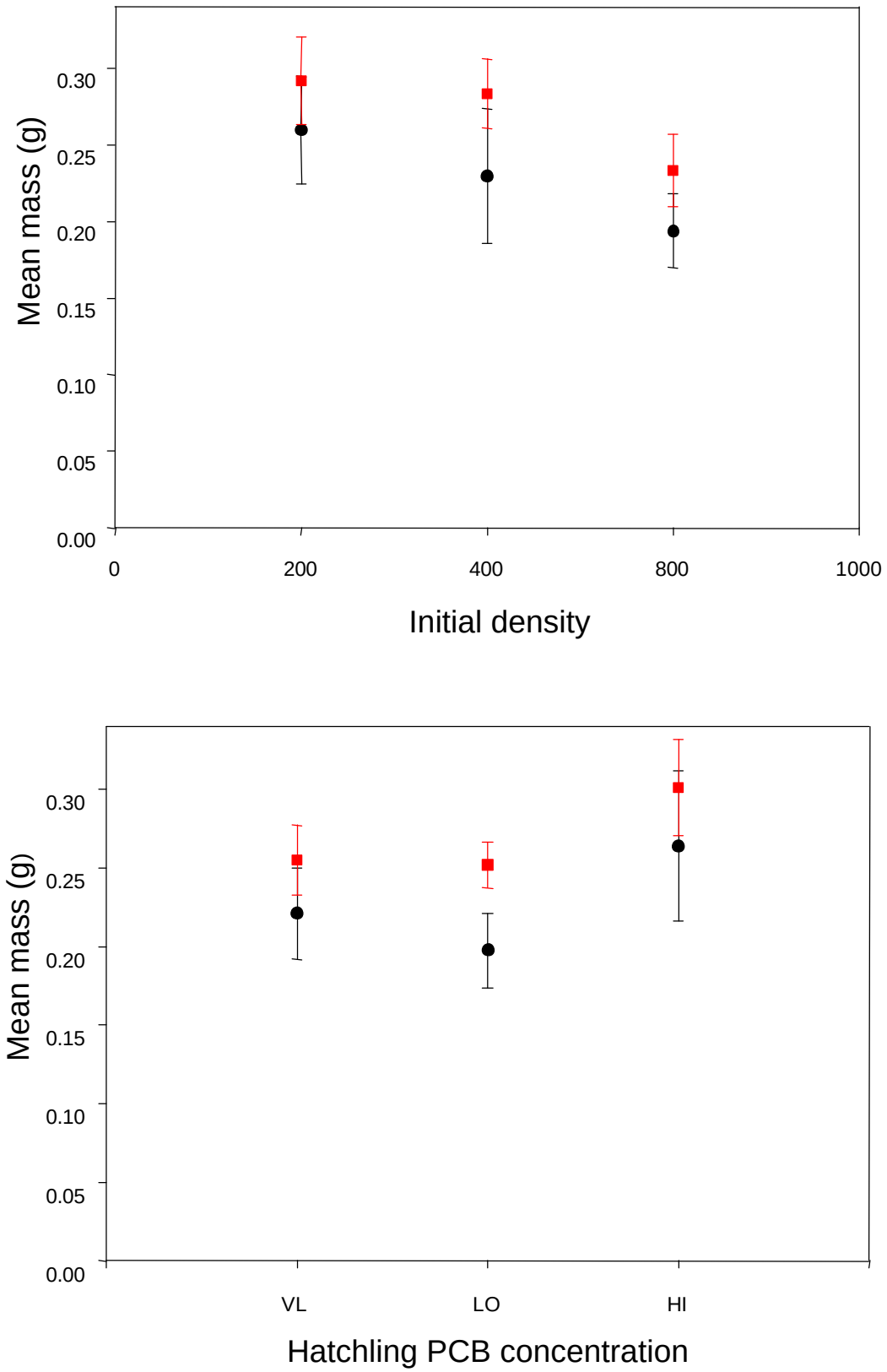


Fig. 6. Mean mass (g) (± 1 SE) of metamorphs (black circles) and surviving tadpoles (red squares) in response to initial density (top) and PCB concentration (bottom).